STUDIES OF THE METABOLISM OF FLAT-SOUR SPOILAGE BACTERIA

BY

DUANE THAYER MAUNDER

B.S., University of Illinois, 1947
M.S., University of Illinois, 1948

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1950

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STUDIES OF THE METABOLISM OF FLAT-SOUR SPOILAGE BACTERIA

Introduction

Thermophilic bacteria concerned with the spoilage of canned foods are generally members of one of three groups, namely flat-sour spoilage bacteria, sulfide spoilage bacteria, or thermophilic anaerobes not producing hydrogen sulfide. This study deals only with organisms that are members of the first-mentioned group.

Flat-sour food spoilage is a condition occurring mainly in the low acid-type canned vegetables. However, one species of this group produces a condition in acid foods, mainly tomato juice, that is spoken of as "flat-sour" spoilage. The spoilage is characterized by a nongaseous acid fermentation without a marked change in odor.

The flat-sour group is comprised of two species — *Bacillus stearothermophilus* and *Bacillus thermoacidurans*. Since little appears in the literature on the nutrition of these organisms, it is the purpose of this investigation to study the growth of the members of this group, paying particular attention to the nutritive requirements of the organisms with respect to vitamins and nitrogen, and to show which acids, as metabolic products, are produced in the fermentation of dextrose.

Experimental and Discussion

Bacillus stearothermophilus, the typical flat-sour food spoiling organism, was first isolated from spoiled canned corn by Donk (1920). A second causative organism was isolated from spoiled tomato juice by Berry (1933) and named *Bacillus thermoacidurans.* This organism and *Bacillus coagulans,* of importance in the dairy industry, are now considered to be identical.

Bacillus thermoacidurans produces acids more rapidly, and

normally exhibits greater resistance to acidity than does *Bacillus stearothermophilus.* However, under conditions in which the acids of metabolism were regularly neutralized before the critical pH's were reached, both species utilized equal quantities of dextrose with the formation of nearly equivalent concentrations of acids.

VITAMIN REQUIREMENTS — The flat-sour organisms have similar vitamin requirements. All strains of both species studied required biotin and thiamin for maximum growth and acid production. In addition, *Bacillus stearothermophilus* required nicotinic acid while *Bacillus thermoacidurans* required the presence of p-aminobenzoic acid for optimal growth. Cleverdon, Pelczar, and Doetsch (1949) previously reported that nicotinic acid, biotin, and thiamin were required for optimal growth of *Bacillus stearothermophilus* 1518.

AMINO ACID REQUIREMENTS —-Study of the amino acid requirements of the flat-sour group showed that several combinations of amino acids (one milligram of l-form or two milligrams of dl-form) supported optimal growth and acid production. Strain lb of *Bacillus thermoacidurans* grew well on a basic mixture of threonine, aspartic acid, methionine, phenylalanine, cystine, valine, glutamic acid, hydroxyproline, and lysine supplemented with any one of the following groups: alanine; alanine and norleucine; leucine, isoleucine, and alanine; or glycine and norleucine.

Strain 43P of *Bacillus thermoacidurans* grew on continued subculture in a medium containing threonine, alanine, histidine, aspartic acid, methionine, glutamic acid, serine, phenylalanine, valine, and isoleucine.

Bacillus stearothermophilus 1518 attained maximum levels of growth and acid production in a medium containing valine, cystine, isoleucine, glutamic acid, threonine, methionine, lysine, serine, alanine, leucine, and histidine.

The presence of ammonium sulfate (0.1%) did not give increased growth of any of the organisms tested.

ACIDS PRODUCED IN THE METABOLISM OF FLAT-SOUR ORGANINSMS — Both synthetic and semi-synthetic media were used in this part of the investigation — the synthetic media contained amino acids, vitamins, dextrose, buffer, and inorganic salts, whereas the semi-synthetic media contained casein hydrolysates and yeast extract in lieu of pure amino acids and vitamins. Incubations were at 55°C for periods varying from twenty-four hours to one week. Separate sterilization of dextrose and its aseptic addition to the other consitituents was found to promote a higher level of acid production than was otherwise possible with *Bacillus stearothermophilus*.

Five acids were shown to be present in metabolic solutions of the strains examined — lactic, formic, acetic, butyric, and propionic acids. Lactic acid, as determined by the method of Barker and Summerson (1941), represented approximately 90% of the total weight of acids produced.

Formic acid was determined by a procedure incorporating features of methods described by Osburn, Wood, and Werkman (1933) and Lenoël (1948). Six to eight percent (by weight) of this acid was found.

Acetic acid was determined by the partition method of Osburn, Wood, and Werkman (1936), two to four percent (by weight) being found.

Less than 0.1% of the total weight of acids produced was found to consist of propionic and butyric acids. These could not be determined separately by the partition method used. A microscopic method of crystal examination described by Ramsey and Patterson (1945) was used to identify these acids.

The production of lactic acid by flat-sour type organisms has been previously reported by Sarles and Hammer (1932) and Pan, Patterson, and Johnson (1940). Although no reference to the information was given, a review by Gaughran (1947) indicated that lactic, formic, and acetic acids are produced by flat-sour bacteria. With respect to volatile acids,

Sarles and Hammer also reported that their results "suggest a mixture of acetic and propionic acids, while Duclaux values indicate largely acetic acid."

Summary

The metabolism of bacteria causing flat-sour spoilage in canned foods was studied. Vitamin and amino acid requirements were determined for the causative organisms, *Bacillus stearothermophilus* and *Bacillus thermoacidurans*. It was found that a mixture of nicotinic acid, thiamin, and biotin was required for optimal growth and acid production on continued subculture at 55°C by *Bacillus stearothermophilus*. A mixture of eleven amino acids permitted optimal growth of this organism at 55°C — valine, cystine, isoleucine, glutamic acid, threonine, methionine, lysine, serine, alanine, histidine, and leucine. *Bacillus thermoacidurans* (lb) required p-aminobenzoic acid, thiamin, and biotin for maximum growth and acid production together with the following amino acids: alanine, threonine, aspartic acid, methionine, phenylalanine, cystine, valine, glutamic acid, hydroxyproline, and lysine. *Bacillus thermoacidurans* (43P) had the same vitamin requirements as strain lb. A mixture of glutamic acid, serine, phenylalanine, valine, isoleucine, threonine, alanine, histidine, aspartic acid, and methionine gave good growth and acid production of this organism at 55°C.

The mixture of acids produced in the metabolism of the flat-sour organisms consists largely of lactic acid with smaller concentrations of formic and acetic acids, and traces of butyric and propionic acids.

BIBLIOGRAPHY

Barker, J. B. and W. H. Summerson. 1941. Iowa State Col. J. Sci., 14, 187-191.

Berry, R. N. 1933. J. Bact., 25, 72-73.

Cleverdon, R. C., M. J. Pelczar, Jr., and R. N. Doetsch. 1949. J. Bact., 58, 523-526.

Donk, P. J. 1920. J. Bact., 5, 373-374.

Gaughran, E. R. L. 1947. Bact. Revs., 11, 189-225.

Lenoël, C. P. 1948. Ann. de l'institut Pasteur, 74, 345-346.

Osburn, O. L., H. G. Wood, and C. H. Werkman. 1933. Ind. Eng. Chem., Anal. Ed., 5, 247-250.

Osburn, O. L., H. G. Wood, and C. H. Werkman. 1936. Ind. Eng. Chem., Anal. Ed., 8, 270-275.

Pan, S. C., W. H. Patterson, and M. J. Johnson. 1940. Ind. Eng. Chem., 32, 709-714.

Ramsey, L. L. and W. I. Patterson. 1945. J. Assoc. Official Agr. Chem., 28,644.

Sarles, W. B. and B. W. Hammer. 1932. J. Bact., 23, 301-314.

VITA

Duane Thayer Maunder was born at Joliet, Illinois on July 27, 1922. His elementary education was received in Minooka, Illinois. He attended Minooka Consolidated High school and was graduated from Joliet Township High school in 1940. In 1942, he was graduated from Joliet Junior College. After one semester at the University of Illinois, he went on active duty with the U. S. Naval Reserve in April 1943.

He served as a Fire Controlman aboard the U. S. S. Canberra (CA-70) in the Pacific theater. He received a commission in November 1945 and was released from active duty in June 1946. At present he is attached to Volunteer Reserve Research Unit 9-2 as a Lieutenant (junior grade).

After returning to the University of Illinois in September 1946, he received the degree of Bachelor of Science and Master of Science in Bacteriolgy in 1947 and 1948 respectively.

From June 1948 to June 1950, he was a teaching assistant in Bacteriology in the College of Liberal Arts and Sciences at the University of Illinois.

He is a member of the Society of American Bacteriologists, Society of Illinois Bacteriologists, Phi Sigma, Sigma Xi, and Pi Kappa Alpha.